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Research Paper

Role of MicroRNAs in the Regulation of Sleep/wakefulness and Their Expression Changes in the Brain Following Sleep Deprivation

Akram Ahangarpour[1], ***Rafie Belali**[2]

1. Department of Physiology, School of Medicine, Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2. Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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ABSTRACT

Background and Objectives The circadian rhythm has 24-hour oscillators and is introduced as an internal sleep/wakefulness cycle regulator. This system is regulated by transcription-translation feedback loops. MicroRNAs (miRNAs) include non-coding RNAs, which are essential in the post-translational modification of mRNA transcripts. MiRNAs play a significant role in circadian rhythm regulation, and it has been demonstrated that sleep loss can alter brain miRNA levels. Nowadays, sleep deprivation has turned into a daunting challenge in modern societies, considering that sleep disorder is the source of many diseases, and numerous studies have examined the association between sleep disorder and miRNA dysfunction. Therefore, it appears that it is critical to assess the relationship between microRNA, circadian rhythm, and sleep disorder.

Subjects and Methods For this purpose, a query was conducted on various databases (Google Scholar, Scopus, Web of Science, and PubMed) for English articles from 1998 to 2021.

Results The current evidence confirms that miRNAs are involved in the molecular regulation of circadian rhythms by regulating sleep duration and intensity. Some of these miRNAs include miRNA-155, miRNA-7b, MiR-182, miRNA-126, miRNA-192/194, miRNA-142-3p, miRNA-132, and miRNA-219-1. Lack of sleep can cause widespread changes in protein expression throughout the brain by altering miRNA (miR-1b, miRNA-125a-3p, miR-146a, miR-26a/b-3p, and miR-138) levels.

Conclusion As reviewed in this study, miRNAs are uniquely expressed at different times and in various structures in the brain, playing a key role in sleep regulation. These findings suggest that understanding the abnormalities in the expression of circadian miRNAs could be used to treat numerous disorders following sleep deprivation.

Keywords Circadian rhythm, Clock gene, MiRNAs, Sleep deprivation, Sleep homeostasis

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**** Corresponding Author:***
Rafie Belali
Address: Department of Physiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Tel: 09179832272
E-Mail: rbelali99@gmail.com

Introduction

In recent years, microarray studies have demonstrated that gene expression varies in different regions of the brain during sleep, wakefulness, and sleep deprivation. Half of the 1,500 genes in the cerebral cortex are expressed differently between day and night due to the switching between sleep and wakefulness [1, 2]. Epigenomics involves chemical variations in the genome that affect the packaging and expression of DNA without changing its sequence. Although it is reversible in response to environmental changes, it leads to persistent and inherited alterations in gene transcription. Epigenetic mechanisms may also regulate circadian rhythms and sleep patterns [2, 3].

1) DNA methylation and hydroxymethylation, 2) histone modification, and 3) non-coding RNA regulation are the three main epigenomic mechanisms [4]. MicroRNAs (miRNAs) are known as an important epigenetic mechanism [5]. MiRNAs are non-protein-coding RNA sequences that act as guide molecules for messenger RNA (mRNA) stability and translation in many biological and pathological processes [6]. MiRNAs are thought to be involved in most basic biological processes, regulating more than 60% of all protein-coding genes [7]. They are short and single-stranded RNAs with 22 nucleotides in length that are processed from 70 nucleotides. 3'untranslated regions (3'UTRs) of mammalian genes contain highly conserved sequences, polyadenylation, and mRNA stability. These functions are mediated by binding to RNA cross-factors, such as miRNAs [8]. It involves pairing between the 3' UTRs of the mRNA and the miRNA 5'-end, inhibiting translation and/or increasing mRNA destruction and/or mRNA disintegration. As a result, they reduce the level of proteins [4]. Nonetheless, some miRNAs have been shown to enhance mRNA stability and translation processes under certain conditions [5]. Each miRNA can target a large number of transcripts and unique mRNAs, including the binding sites of multiple miRNAs, leading to the simultaneous targeting of multiple genes [9].

In the central nervous system, miRNAs are associated with synaptic plasticity in the brain, neurogenesis, and neurite growth. Several reasons prove that miRNAs are promising targets for modulating synaptic junctions: (1) many types of miRNAs are expressed in the brain and have a specific structure. (2) miRNA/mRNA clusters are found at the synapse. (3) Some miRNA species appear to be activity-dependent. (4) miRNAs are related to the RNA-induced silencing complex, a regulator of synaptic protein synthesis associated with memory formation [9,5]. Recent studies have linked miRNAs to sleep-wake regulation. Sleep is crucial in normal biological functions, such as temperature regulation, immune response, and cognitive function, especially during memory consolidation. In today's society, sleep disorder has become a public health problem due to its significant social and economic consequences [10].

sleep disorder generally refers to sleeping less than six hours during 24 hours [11]. Numerous reports indicate that sleep disorder exerts a negative effect on cognitive functions, such as emotional memory, working memory, and hippocampal memory [12]. sleep disorder brings about extensive changes in protein and gene expression in the brain known as sleep regulators, including N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, brain-derived neurotrophic factor (BDNF), cAMP- response element-binding protein (CREB), and c-fos [13,14 ,15]. After sleep disorder, changes in the expression of NMDA receptor subunits (NR1, NR2A, and NR2B) and AMPA receptors could contribute to cognitive decline and the neurodegenerative process [16,17]. Moreover, the gene expression of CREB and BDNF, which are required for memory consolidation and brain development, decreases in the neocortex and hippocampus [18].

The hypothesis that sleep disorder pointed out ads to changes in the expression of brain microRNAs stems from the ability of miRNAs to regulate mRNA expression. Microarray analysis also pointed out that 50 miRNAs were altered after eight hours of sleep disorder [18]. Sleep-related miRNAs regulate sleep by targeting sleep-related mRNAs, and sleep disorders can alter miRNA levels in the brain [19]. In light of the aforementioned issues, this paper aims to review recent studies investigating the role of microRNAs in regulating sleep/wakefulness and their expression

changes in the brain following sleep disorder. At first, we look at the role of miRNAs in regulating sleep and wakefulness, and then we review the studies that have examined the changes of miRNAs in the brain after induction of sleep disorder.

Materials and Methods

We conducted a review of the literature using such keywords as "circadian rhythm," "MicroRNAs," "sleep," "wakefulness," "Sleep homeostasis," sleep loss," "sleep deprivation," "CLOCK⁄BMAL1", "miR-155", "Let-7b", "miR-138"," miR-125a", "miR-126", "miR-192/194", "miR-142-3p", "miR-132", "miR-219-1", "miR-1b"," miR-182", "miR-146a" and "miR-26b-3p". PubMed, Web of Science, Google Scholar, and Scopus databases were searched for articles from 1998 to 2021.

Results

1) Role of microRNAs in the regulation of circadian rhythms

1.1 Circadian rhythms

Sleep homeostasis and circadian rhythm are strongly connected. Sleep disorders are caused by disruptions in the circadian rhythm and vice versa. The first step in this process is to explain the relationship between them [20]. Almost all animals exhibit a wide range of circadian rhythms in behavior and physiology, including motor activity, sleep, learning, memory, mating, and endocrine function [21]. Circadian rhythm (about 24 hours) is regulated by cellular or pacemaker "clocks," which can be synchronized with daily and seasonal changes in zeitgebers, especially visible light and ambient temperature [22]. Daily fluctuations in physiological and behavioral processes can be observed in various organisms.

Oscillating rhythms are produced by an internal clock mechanism called the circadian clock [23]. The suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is referred to as the main pacemaker of the circadian rhythm, which promotes daily behavioral and physiological rhythms. Nevertheless, there is also evidence for circadian oscillators outside the SCN, such as peripheral clocks located throughout the body, that provide local coordination in a specific tissue or cell process in a timely manner [24]. The twenty-four-hour rhythm is regulated by relying on the molecular mechanisms of various clock genes, such as those encoding transcriptional activators, inhibitors, and modifying enzymes.

According to Figure 1, the molecular mechanism of the circadian clock consists of several feedback loop systems, as well as a transcription and translation phase. In mammals, the first loop contains the positive elements of circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-Like 1 (BMAL1) [25]. CLOCK protein is a major component of circadian rhythms with histone acetyltransferase (HAT) activity. The transcription factors CLOCK⁄ BMAL1 heterodimer protein bind to E-boxes and activate the clock genes: *Period* (*Per1* and *Per2*), *Cryptochrome* (*Cry1* and *Cry2*), *D element–binding protein* (*Dbp*), *Rev-erb*, and *retinoic acid-related orphan receptors* (*Ror*), as well as clock-controlled genes, including miRNAs. RORα(*Retinoic acid-related orphan receptor α* (RORα) is a nuclear receptor encoded by the *RORA* gene) acts through amplifiers of the ROR response element (RORE).

The DBP binds to the D-box element in target genes, including *Per1-3*, *Rev-erb,* and *Ror*. The protein products of these genes suppress the CLOCK/BMAL1 complex and also their own expression. After destroying PER and CRY products, CLOCK/BMAL1 will be released [26-28].This feedback loop contains the molecular tool of the circadian timekeeping mechanism, and it is common in the SCN and peripheral cell-autonomous clocks [29].

When BMAL1 negative regulators, PER, and CRY proteins are at their peak in the nucleus, they act as E-box suppressor complexes dependent on gene activation [30]. However, the expression of these proteins is modulated by the interaction of miRNA with the 3'-UTR of these genes [31]. Transcription of genes that have RORE in the region upstream of the promoter, such as *Cry, Bmal1*, *E4bp4*, and miRNAs, is reduced by REV-ERBα. The binding of E4BP4 to the D-box can repress the transactivation of *Per* and *Rev-erb*. Furthermore, miRNAs down-regulate core clock components and modulate circadian rhythm directly. The CLOCK/BMAL1 complex also activates two other feedback loops, including the vrille (VRI) and PAR domain protein 1 (PDP1) feedback loops that modulate CLOCK mRNA and the CLOCKWORK ORANGE (CWO) feedback loop [32, 33 , 34, 35, 36].

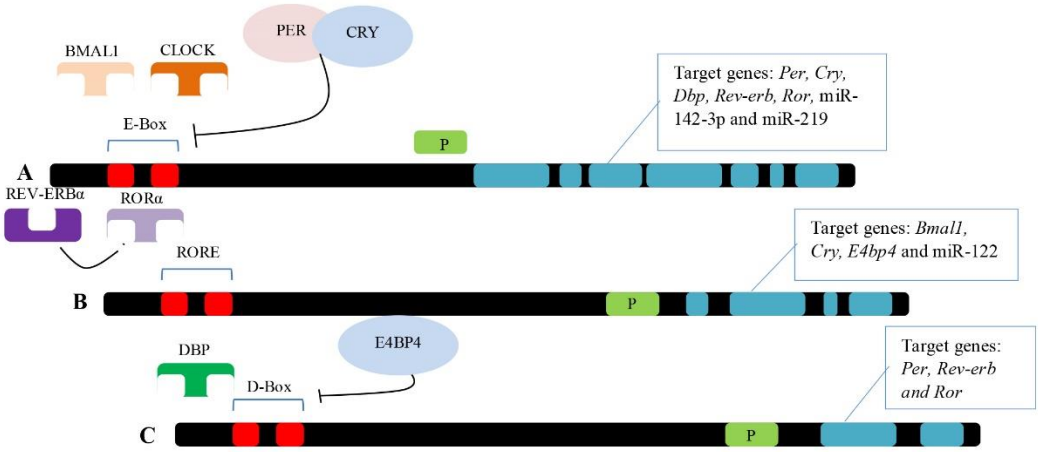

Figure 1: Molecular regulation of core clock genes in the circadian system
A; Pathway 1: CLOCK: BMAL1 transcription factors bind to the target E-Box and activate the clock genes *Per, Cry, Dbp, Rev-erb*, and *Ror*, as well as clock-controlled genes, including miRNAs. After the synthesis of PER and CRY in the cytoplasm, these proteins inhibit E-Box and reduce the expression of target genes. **B**; Pathway2: output genes that have RORE in the upstream region of the promoter, such as *Bmal1, Cry, E4bp4,* and *miR-122*, are inhibited by active RORα and REV-ERBα. **C**; Pathway 3 also shows DBP-dependent activation, which is initiated by binding to the D-box and suppressed by competitive binding of E4BP4. This pathway is responsible for the transcription of *Per, Rev-erb,* and *Ror*. P: promoter.

Recent studies have pointed out that post-transcriptional mechanisms, such as phosphorylation, ubiquitination, sumoylation, and acetylation, are also involved in the modulation of clock proteins and the oscillating behavior of the molecular components of the nucleus [37]. Recently, miRNAs, a new class of posttranscriptional regulators, have been shown to have regulatory functions over circadian rhythms [38]. Some of the miRNAs involved in circadian rhythm regulation are summarized in Table 1.

Table 1. microRNAs involved in regulating circadian rhythms

MicroRNAs	Target clock genes	Function	References
miR-29a/b/c	*Per1* *Per2* *Per3*	MiR-29a/b/c down-regulate hPER1 at both mRNA and protein expression levels in human A549 cells by target hPER1 3'untranslated region (UTR).	(Zhao et al., 2014)
miR-219 miR-132	*Per1* *Per1*	MiR-219 and miR-132 are involved in modulating the circadian clock located in the suprachiasmatic nucleus. MiR-219 is the target of the CLOCK / BMAL1 suite, and miR-132 modulates clock gene expression via MAPK / CREB.	(Cheng et al., 2007)
miR-125a-3p miR-144 miR-199a-5p miR-199b miR-200a miR-200b miR-203 miR-449a miR-96	*Per3* *Clock* *not clear* *Clock* *Clock* *Clock* *Clock* *Per2* *not clear*	The expression of these miRNAs seems to be regulated indirectly by CLOCK in the mouse SCN.	(Wang et al., 2018)
miR-103	*Per3*	The expression of miR-103 could be induced by the clock gene BMAL1 and modulate the circadian regulation in rat cerebral arteries at the posttranscriptional level. Moreover, in colorectal cancer cells, miR-103 regulates the *Per3*.	(Li Chen et al., 2019; Hong, Feng, Sai, & Tao, 2014)
miR-34a-5p	*Per1* *Per2*	There is a negative correlation between *PER2* mRNA and miR-34a expression in patients with more advanced cancer.	(Hasakova, Reis, Vician, Zeman, & Herichova, 2019)
MiR-122	*not clear*	PPARs might act as mediators to link miR-122 function to the control of circadian gene expression and hepatic lipid metabolism	(Gatfield et al., 2009)
miR-24 miR-30a	*Per2*	MiR-24 and miR-30a increase the expression of *Per2* and other clock genes.	(Yoo et al., 2017)

Table 1 Continue

miR-279	*upd*	The central clock controls sleep/ wake rhythms by regulating JAK/STAT signaling. PER and *upd* are co-expressed; mir-279 regulates *upd* and acts downstream of the clock gene, and targets the JAK/STAT.	(Luo & Sehgal, 2012)
miR-152 miR-494	*Bmal1* *Bmal1*	The circulating levels of miR-494 and miR-152 regulate core clock components. They exhibit an interesting relationship with reported circadian profiles for Bmal1 in the periphery.	(Shende, Goldrick, Ramani, & Earnest, 2011)
miR-135b	*Bmal1*	MiR-135b suppressed BMAL1 expression directly targets the BMAL1 3′-UTR and Forms miR-135b–BMAL1–YY1 loop as a determinant of pancreatic circadian homeostasis.	(W. Jiang et al., 2018)
miR-211	*Bmal1* *Clock*	Bmal1 and Clock, core circadian regulators, are suppressed transiently by miR-211, which is a PERK inducible micro-RNA.	(Chitnis et al., 2012)
miR-27b-3p	*Bmal1*	MiR-27b-3p targets the clock gene Bmal1 at the posttranscriptional level in the mouse liver.	(Zhang et al., 2016)

1.2 MiR-155

Many physiological parameters, including the immune response to infectious agents, are controlled by the circadian clock, which is activated by the transcription nuclear factor kappa B (NF-κB). It is widely accepted that circadian regulation is based on periodic changes in gene expression, which are caused by the transcriptional activity of the CLOCK/BMAL1 complex. Generally, CLOCK has been considered a key component of circadian activation that is dimerized with BMAL1 to stimulate the rhythmic expression of E-box-containing promoters. CLOCK is also introduced as a modulator of NF-κB transcriptional activity. In the absence of BMAL1, NF-κB-mediated transcriptional up-regulation can be performed by CLOCK, which is a core circadian protein. BMAL1 also counteracts the CLOCK-dependent increase in the activation of NF-κB-responsive genes [39].

MiR-155 is an important regulatory component of circadian function, which can be altered by controlling inflammatory cytokines and affecting BMAL1. MiR-155 has been identified as an important suppressor after Bmal1 transcription. High levels of miR-155 may lead to a pro-inflammatory state by activating the NF-κB complex. MiR-155 controls circadian inflammatory responses by targeting Bmal1. On the other hand, Bmal1 can stop the inflammatory pathway by interfering with miR-155 activation [40]. Knockout miR-155 in mice reduced non-rapid eye movement (NREM) and rapid eye movement (REM) sleep effort compared to healthy mice, but no difference was observed during sleep or in the total time spent sleeping and waking [41]. Sleep disorder increases miR-155 and inflammatory cytokines [42].

1.3 Let-7

The human lethal-7 (let-7) family of miRNAs contains 12 members [43]. Let- 7 miRNA acts as a circadian rhythm regulator. Excessive expression of let-7 in clock neurons prolongs the circadian cycle, and its elimination weakens the peak of morning activity and molecular oscillation. Let-7 regulates circadian rhythms by suppressing the CWO feedback loop [44]. At the end of the dark period, the expression of this miRNA is increased in the prefrontal, occipital, and somatosensory cortex more than at the end of the light period. An intraventricular use of a let-7b-specific antiMIR in rats does not affect the duration of NREM and REM sleep in the light phase; nonetheless, it significantly reduces the potency of EEG delta waves in NREM [45]. After sleep disorder, let-7b levels upregulate in the hippocampus, and down-regulation occurs in the hypothalamus, prefrontal, and somatosensory cortices [46]. Plasma levels of let-7f also increase with miR-30c and miR-26a in patients with narcolepsy and other central hypersomnia [47]. The down-regulation of let-7 levels has been observed in patients with obstructive sleep apnea. This miRNA targets the CNR1 and CRY2 genes [48]. The family of the let-7 (a-f) acts as a supplement with the leading causes of sleep regulators, such as Tumor necrosis factor α (TNFα), IL-1, and IkB, an inhibitor of NF-κB [49].

1.4 MiR-182

The suprachiasmatic nuclei (SCNs) of the hypothalamus have a master clock that controls the circadian rhythm. As mentioned earlier, miR-132 and miR-219-1 are circadian clock modulators in the SCN [50]. The miR-183/96/182 cluster is also involved in regulating circadian rhythms by targeting the CLOCK, regulatory factor X4 (RFX4), PH domain leucine-rich repeat protein phosphatases (PHLPP), and adenylate cyclase 6 (ADCY6) genes. MiRNAs, especially miR-182,

make a precise adjustment after transcription in the circadian rhythm, targeting the precursor rs76481776 SNP (Single nucleotide polymorphism), which is involved in the control of sleep and wakefulness [50. MiR-182 is involved in the regulation of the clock gene in the pineal gland. Moreover, it protects the optical receiver of the outer layer cone and its visual function. Previous studies have demonstrated that abnormal processing of pre-miR-182 may lead to the dysregulation of circadian rhythms in patients with major depression associated with insomnia [51].

1.5 MiR-126

MiR-126 is crucial for proper endothelial function and vascular homeostasis [53]. Knocking out miR-126 leads to a loss of vascular integrity and impaired endothelial cell proliferation in vivo. Phospho-inositide 3-kinase (PI3K)-dependent Protein Kinase B (Akt) activation causes Bcl-2 agonist of cell death (BAD) phosphorylation in Ser136/Ser112. BAD is a BH3-only member of the Bcl-2 family [54].

Phosphorylated Bad is depolymerized from apoptosis regulators, such as B-cell lymphoma-2 (Bcl-2) or B-cell lymphoma-extra-large (Bcl-xL), acting as an anti-apoptotic factor by interacting with 14-3-3 proteins. 14-3-3 proteins are a group of conserved regulatory proteins found in all eukaryotic cells. Meanwhile, Bcl-2 disintegration can also act as an anti-apoptotic protein. PIK3R2 is the direct target of miR-126, known as the PI3K/Akt signaling pathway suppressor.

The overexpression of miR-126 results in the suppression of apoptosis by increasing activation of the anti-apoptotic pathway PI3K/Akt [55]. MiR-126 activates endothelial nitric oxide synthase (eNOS). The miR-126 mimic increases nitric oxide (NO), vascular endothelial growth factor (VEGF), superoxide dismutase (SOD) content, and cell proliferation by increasing the expression of p/t-PI3K, p/t-Akt, and p/t-eNOS. It also reduces reactive oxygen species (ROS) content and the expression of IL-6, IL-10, and TNF-α 56]. This miRNA decreased in adults who slept less than seven hours a night, and it is effective in causing vascular disorders by reducing the proliferation of endothelial cells [56].

1.6 MiR-192/194

The expression of miR-192/194 affects the circadian cycle by down-regulating all members of the per gene family. The elimination of the Per1, Per2, and Per3 genes in mice shortens the circadian period by about 1, 1.5, and 0.5 hours, respectively. Observations demonstrate that miR-192 and miR-194 are both expressed in the liver and kidney. This finding is interesting since both of these tissues can maintain circadian rhythms without SCN function [57]. Hepatocyte nuclear factor-1a (HNF-1alpha) and p53 can induce miR-192/194 [58, 59].

1.7 MiR-142-3p

The expression of miR-142-3p in SCN results in molecular clockwork integration. MiR-142-3p modulates Bmal1 expression in the SCN of mice, playing a major role in the circadian control of the clock gene. The overexpression of this miRNA disrupts the rhythm of BMAL1 protein accumulation since Bmal1 is widely expressed in most cells and tissues of the body and is regulated rhythmically [60]. MiR-142-3p acts as a suppressor after the transcription of Bmal1 in SCN. MiR-142-3p levels peak at the subjective beginning of the day while Bmal1 expression decreases. Indeed, miR-142-3p-mediated BMAL1 activity suppression is required to maintain the circadian rhythm, demonstrating the importance of transcriptional feedback in mammalian clock function [61]. MiR-142-3p is an extracellular monitoring signal that is involved in the posttranscriptional modulation of the molecular nucleus of clockworks. Therefore, these and other miRNAs that target the nucleus of clock genes may act as cis- and trans-signals that locally regulate or coordinate the timekeeping components of the circadian rhythm between the peripheral cell-independent clocks [62].

1.8 MiR-132 and miR-219-1

MiR-132 is a mediator of synaptic plasticity, as its values change with decreasing sleep and time of day. In addition, miR-132 is associated with circadian rhythms, dendritic modifications, and the sleep regulation process. The gene expression of this miRNA is strongly regulated by light and plays a crucial role in the photic entrainment of circadian rhythms, a process in which the SCN coordinates with a 24-hour cycle of dark and light. Long-term memory improvement requires the phosphorylation of the CREB transcription factor by the microRNA-132

promoter. Furthermore, miR-219-1 modulates course length in SCN. The Rfx4 and Phlpp genes are the targets of miR-132 and miR-219-1 in SCN, respectively. The miR-219 and miR-132 can shorten the circadian cycle and negatively affect clock light settings [50]. The effects of miR-132 are at their peak on mPer1 transcriptional level and PER2 protein expression, thereby affecting the core of the scheduling process [50].

Intraventricular injection of a miR-132 mimic (preMIR-132) reduced NREM sleep duration and increased REM sleep duration in the light phase. In addition, preMIR-132 reduces the activity of slow electroencephalographic waves during NREM sleep, which is an indicator of sleep intensity [19, 45]. After sleep disorder, it decreased in both the cortex and hippocampus [5]. The miR-219 level reaches its peak from the beginning to the middle of the day and supports the CLOCK/BMAL1-dependent transcription rhythm. This time window coincides with the maximum expression of mPer1. Low expression of miR-219 occurs at night. When PER/CRY protein complexes reach sufficient levels, they inhibit CLOCK/BMAL1-mediated transcription and reduce miR-219 expression [63].

2) MiRNAs associated with sleep deprivation

2.1 MiR-1b

It has been illustrated that some miRNAs are outside the cells, and they can be found in body fluids. MiR-1 is one of the miRNAs that can be transported through the blood. It has been demonstrated that miR-1 is expressed in the heart, muscles, and other tissues; however, it appears to be secreted into the bloodstream and acts on hippocampal neurons. After 72 hours of sleep disorder, pri-miR-1b levels in the hippocampus decreased. Therefore, sleep disorder decreases the regulation of miR-1 in the hippocampus, which is associated with impaired cognition, memory, and learning [12]. MiR-1 expression is positively correlated with changes in BDNF mRNA in the hippocampus [64]. An in vitro study on cell culture reported that miR-1B pointed out that miR-1b could directly inhibit BDNF expression in different cell types by targeting the 3'-UTR and also regulate Schwann cell function by suppressing BDNF expression in peripheral nerve damage [65].

2.2 MiR-125a

MiR-125a plays a key role in the pleiotropic promoter of endothelial health and vasomotor function. Changes in circulating levels of this miRNA are associated with vascular inflammation, endothelial dysfunction, and the prognosis of cardiovascular disease and mortality [53]. Sleep disorder causes a decrease in circulating miR-125a levels. MiR-125a directly targets the 3'UTR mRNA endothelin-1 (ET-1) and inhibits its translation. ET-1 is a vasoconstrictor that increases after sleep disorder. Therefore, a decreased expression of miR-125a is associated with increased production and secretion of ET-1 [66,53]. MiR-125a targets the casein kinase I (CKI) and Per3 genes, thereby being involved in long-term sleep regulation [20].

At the end of the dark period, compared to the end of the light period, the expression of this miRNA increases in the prefrontal cortex, occipital cortex, and somatosensory cortex. An intraventricular injection of a specific anti-MIR miR-125a in the rat has no effect on the strength of EEG delta waves in NREM; nonetheless, it can reduce NREM time in the light period and increase it in the dark period [19]. After sleep disorder, there is an increase in the hippocampus and a decrease in the hypothalamus, the peripheral cortex, and the somatosensory cortex [9].

2.3 MiR-146a

MiR-146a is an essential inflammatory modulator in the frontal cortex. Total sleep deprivation(REM and NREM sleep deprivation) (TSD) causes the overexpression of miRNAs associated with the inflammatory process. 24-hour sleep deprivation increases miR-146a levels in the frontal cortex, and these changes persist even after a six-hour recovery period [67].

In addition to miR-146a, miR-155, -223, -16, -126, and -21 have been identified as potential biomarkers of inflammatory processes. Acute paradoxical sleep deprivation(REM sleep deprivation) (PSD) increases the levels of inflammation-related molecules, including IL-6, miR-146a, miR-155, and miR-223. Moreover, it decreases the levels of the anti-inflammatory cytokine IL-10 [42]. Nevertheless, a study on adults who chronically slept less than seven hours a night illustrated that circulating levels of miR-125a, miR-126, and miR-146a decreased. These changes are related to

vascular inflammation and dysfunction. Furthermore, they increase the incidence of cardiovascular disease (Hijmans et al., 2019).

It has also been found that miR-146a levels in heart tissue after myocardial infarction (MI) are associated with its severity and complications. Patients with cyanotic congenital heart disease and chronic hypoxia displayed increased miR-146b-5p in cardiac tissue, suggesting a protective role for miR-146b in this disease. MiR-146a is an NF-κB-dependent gene that acts as an endotoxin-responsive gene in human monocytes, along with miR-146b, miR-132, and miR-155 [68]. The increased expression of these miRNAs is part of the body's rapid response to oxidative stress and endotoxemia caused by sleep deprivation [69].

MiR-146a/b appears to be able to control Toll-like receptors and cytokine signaling via a negative feedback regulation loop that includes the down-regulation of IL-1 receptor-associated kinase 1 and TNF receptor-associated factor 6 (TRAF6) protein levels 68]. TRAFs are adaptor proteins that play a critical role in intracellular signaling, mediating signals that are essential for the homeostasis, development, and activation of B, T, and myeloid cells [70, 71]. Therefore, the overexpression of miR-146a reduced the mRNA and protein levels of TRAF6 and NF-κB, as well as inflammation.

2.4 MiR-26a/b-3p

MiR-26b-3p is one of the circulating miRNAs associated with sleep duration and circadian rhythm in humans. This miRNA targets several genes, including *Protein kinase AMP-activated catalytic subunit alpha 2* (*PRKAA2*), *CREB1*, *Casein kinase 1 delta* (*CSNK1D*), and *RORA*. These genes are involved in the regulation of circadian rhythms, the regulation of apoptosis, and the dynamics of microtubules. MiR-26b-3p and miR-485-5p target the 2-phosphoglycerate enolase (ENO1) gene, which is involved in regulating several energy source pathways, such as glycolysis/gluconeogenesis, carbon metabolism, biosynthesis of amino acids, RNA degradation, and the HIF-1 signaling pathway [3]. In addition, studies pointed out that plasma miR-26a levels are impaired in patients with narcolepsy and idiopathic insomnia [72]. MiR-26a inhibits DBP and reduces the expression of the *Per* and *Rev-erb* genes [20]. MiR-485-5p is also down-regulated in the serum of people with obstructive sleep apnea, a sleep-related respiratory disease, and this miRNA potentially participates in the cellular response to hypoxia and the HIF-1α signaling pathway [73]. Chronic sleep deprivation lowers miR-26a/b-3p levels and inhibits PRKAA2, CREB1, CSNK1D, RORA, and DBP. This appears to be an attempt to increase the duration of sleep [3, 20].

Table 2. microRNAs changes following sleep deprivation.

MicroRNAs	Sleep Deprivation Type	Result	References
miR-10B	Sleep deprivation tank	Sleep deprivation for 48 hours decreases miR-10B.	(Y. Jiang & Zhu, 2015)
miR-26b-3p miR-485-5p	short sleepers children <13 children >13	Circulating the levels of miR-26b-3p and miR-485-5p were decreased in children <13 years who slept less than nine hours per day and Children >13 who slept less than eight hours per day.	(Iacomino et al., 2020)
miR-34a miR-92a miR-125a miR-126 miR-145 miR-146a miR-150 miR29c miR-151 miR-212 miR-410	sleep <7 h night	Chronic short sleep is associated with a marked reduction in circulating levels of miR-125a, miR-126 and miR-146a. There were no difference in the Circulation levels of miR-34a, miR-92a, miR-145, and miR-150.	(Hijmans et al., 2019)
let-7e miR-30d miR -10 miR-107 miR -181a let-7b miR-125a miR -128b	gentle handling	After sleep-deprived for 8 h, let-7e, miR-30d, -103, -107, and -181a significantly up-regulated and let-7b, miR-125a -128b significantly down-regulated	(Christopher J Davis et al., 2007)
miR-191a	Paradoxical sleep deprivation by multiple platforms	Paradoxical sleep deprivation for 72 h increased miR-191a in the ovariectomized rats to regulate BDNF levels.	(Mohammadipoor-Ghasemabad et al., 2019b)

Table2 Continue

miR-146a miR-155 miR-223 miR-16 miR-126 miR-21	Paradoxical sleep deprivation by modified multi-platform	24 h PSD increased miR-146a, miR-155, miR-223, and miR-16 serum levels. 96 h PSD decreased miR-223 and 192 h PSD decreased miR-16 and miR-126. After 20 days of recovery, miR-21 was increased and miR-16 was decreased in serum.	(Brianza-Padilla et al., 2018)
miR-152 miR-361 miR-17 miR-18a miR-19a miR-20a miR-19b-1 miR-92a-1	Gentle handling	Sleep-deprivation in mice (4 or 6h) down-regulates miR-152, miR-361, miR-17, miR-18a, miR-19a, miR-20a, 19b-1,and miR-92a-1	(Rolls et al., 2015)

Glossary: sleep deprivation tank: Two small platforms placed in water tanks with 30 cm × 30 cm × 30 cm emerge from and submerged by water alternatively and continuously. Therefore, it forces the animal to move constantly to avoid contact with water. **Paradoxical sleep deprivation**: A small circular platform (such as an inverted pot) surrounded by water. Muscle atony in REM sleep causes the animal to fall into the water. **Gentle handling**: Keep the animal awake with light sounds, shake the cage gently, or make direct contact with the brush or hand(COLAVITO et al., 2013).

2.5 MiR-138

It has been demonstrated that miR-138 is expressed only in the brain and plays an important role in brain development and function. *Hsa-miR-138-5p* plays a crucial role in the processes related to the performance of episodic memory in humans [74]. At the end of the dark period, the expression of this miRNA increases in the hippocampus, hypothalamus, prefrontal, somatosensory, and occipital cortex compared to the end of the light period. Its highest levels were observed in the hippocampus and hypothalamus shortly before the onset of the light period. An intracerebroventricular injection of a specific miR-138 inhibitor (antiMIR) in rats reduces sleep duration, the slow-wave activity of NREM, and the delta power of EEG waves in NREM (an electrophysiological marker used as an indicator of sleep intensity) during the light period [13, 19]. After sleep disorder, there is a marked increase in regulation in the hippocampus and a decrease in regulation in the hypothalamus, peripheral cortex, and somatosensory cortex [75]. A number of studies that have examined the changes in miRNAs after SD are presented in Table 2.

Discussion

As mentioned before, miRNAs have one of the most crucial mechanisms for regulating posttranscriptional gene expression. They are a class of short, non-coding RNAs, which are approximately 20 nucleotides in length. A large number of miRNAs are involved in the molecular mechanism of the Circadian rhythm. Therefore, they are able to adjust the duration and intensity of sleep. Sleep disorder has become commonplace in modern society, causing widespread changes in protein expression throughout the brain by altering the levels of miRNAs. Some miRNAs are down-regulated after sleep disorder, and some are upregulated or both can occur in different areas of the brain. In recent years, they have been used as a marker in the early diagnosis of some diseases associated with sleep disorders, which have been attributed to the role of miRNAs in the regulation of sleep/wakefulness. Considering all of this evidence, it seems that more cellular, experimental, and clinical studies are needed to understand the exact role of miRNAs in sleep homeostasis.

List of abbreviations

ADCY6: Adenylate cyclase six genes

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

Akt: Protein kinase B

Bad: BCL2 Associated agonist of cell death

Bcl-2: B-cell lymphoma 2

Bcl-xL: B-cell lymphoma-extra large

BDNF: Brain-derived neurotrophic factor

BMAL1: Brain and muscle ARNT-Like 1

CLOCK: Circadian locomotor output cycles kaput

CKI: Casein kinase I gene

CREB: cAMP- response element-binding protein

Cry: Cryptochrome gene

CSNK1D: Casein kinase 1 delta gene

CWO: Clockwork orange

DBP: D-Box binding PAR BZIP

EEG: Electroencephalography

ENO1: 2-phosphoglycerate enolase gene

eNOS: Endothelial nitric oxide synthase

ET-1: Endothelin-1

FBXW11: F-Box and WD repeat domain containing 11 gene

HAT: Histone acetyltransferase

HIF-1: Hypoxia-inducible factor1

IkB: Inhibitor of nuclear factor kappa B

IL-1: Interleukin-1

JAK/STAT: Janus kinase-signal transducer and activator of transcription

MAPK: Mitogen-activated protein kinase

MI: myocardial infarction

MiRNAs: MicroRNAs

NF-Kb: Nuclear factor kappa B

NMDA: N-methyl-D-aspartate

NO: Nitric oxide

NREM: Non-rapid eye movement sleep

PDP1: PAR domain protein 1

Per: Period gene

PHLPP: PH domain leucine-rich repeat protein phosphatases gene

PI3K: Phosphoinositide 3-kinases

PRKAA2: Protein kinase AMP-activated catalytic subunit alpha 2 gene

PSD: Paradoxical sleep deprivation

REM: Rapid eye movement sleep

RFX4: Regulatory factor X4 gene

RORA: Retinoic acid-related orphan receptor A

RORE: ROR response element

RORs: Retinoic acid-related orphan receptors

ROS: Reactive oxygen species

SCN: Suprachiasmatic nucleus

SD: sleep deprivation

SNP: Single nucleotide polymorphism

SOD: Superoxide dismutase

TNFα: Tumor necrosis factor α

TRAF6: TNF receptor-associated factor 6

TSD: Total sleep deprivation

3'UTRs: 3'untranslated regions

VEGF: Vascular endothelial growth factor

VRI: VRILLE is a bZIP transcription factor

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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